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THE NEUROENDOCRINE REGULATION OF PATERNAL CARE IN THE SIBERIAN DWARF HAMSTER, PHODOPUS SUNGORUS

by

Krystal Lynn Murphy
2nd Lieutenant, USAF

Master of Science
Arizona State University
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ABSTRACT

This set of experiments was conducted to explore the physiological regulation of paternal care. In the Siberian dwarf hamster (Phodopus sungorus), males may fill two important parental roles 1) nurturing the young, and 2) defending the young from conspecifics. Both are uniquely coupled to the paternal state, and so, may share a common underlying mechanism. Other studies of this species suggest that the hormone prolactin (PRL) might be instrumental in such a mechanism. In fact, paternal nurturing appears to be dependent on plasma levels of PRL. Paternal aggression, however, could be a simple stimulus-response to the female and pups rather than a product of an enduring mechanism. I tested this possibility by observing the response of parental males to an intruder in both the presence and absence of the family group. Paternal aggression was not dependent on the presence of a "stimulus." Interestingly, aggression was not coupled to plasma PRL either. An alternative hypothesis was that PRL mediated the components of paternal care through separate actions. In P. sungorus, the male's plasma PRL steadily increases during his mate's pregnancy. Perhaps this is a critical period for hormonal action. Thus, while nurturing behaviors may be activated by PRL, aggressive behaviors may be organizationally "programmed" by the hormone. To test these hypotheses, I suppressed the males' plasma PRL with a pharmacological agent throughout the time they were paired with females. The results verified that pup-nurturing behavior is directly activated by PRL, while paternal aggression is not directly dependent on PRL for either its activation or organization.

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THE NEUROENDOCRINE REGULATION OF PATERNAL BEHAVIOR IN THE SIBERIAN DWARF HAMSTER *PHODOPUS SUNGORUS*

by

Krystal Lynn Murphy

A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science

ARIZONA STATE UNIVERSITY

August 1991

THE NEUROENDOCRINE REGULATION OF

PATERNAL BEHAVIOR IN THE SIBERIAN DWARF HAMSTER

PHODOPUS SUNGORUS

by

Krystal Lynn Murphy

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ABSTRACT

In the Siberian dwarf hamster Phodopus sungorus, parental males nesting with young are known to be more aggressive than are unmated males. Defense from conspecifics could be a paternal role. As paternal behavior, paternal aggression might be expected to share a common mechanism with the other paternal behaviors, such as nesting and cuddling. An alternate explanation of this increased aggressive behavior is that aggression is stimulated in the parental males by the presence of a defendable resource (the female and pups). To test whether or not paternal aggression results from an internal, physiological mechanism rather than a stimulus response, I tested parental males with a male intruder in both the presence and absence of the family group. Parental males were consistently and significantly more aggressive than control males, regardless of the presence or absence of the family group. Thus, paternal aggression appears to result from a physiological program. The question then arises as to what regulates this behavior. Other studies in this species suggest that paternal care is correlated with the hormone prolactin (PRL). Paternal aggression does not appear to be due to an activational effect of PRL since aggressive behavior persisted despite a significant

decrease in plasma PRL. The enduring nature of aggressive behavior suggests it arises through a neuroendocrine "program." Evidence from other studies suggests that plasma levels of prolactin increase in males of this species during the time of their mate's pregnancy. PRL may be mediating the components of paternal behavior in two different manners. Pup nurturing behavior may result from an activational effect of PRL, while paternal aggression may result from an organizational effect during the mate's pregnancy. To test these hypotheses, I injected males with an inhibitor of PRL throughout the time period they were paired with a female and quantified nurturing and aggressive behavior. Results indicate that parental males with suppressed PRL decrease the frequency of nurturing behavior but the paternal aggressive behavior persists. Thus, pup-nurturing behavior appears to be due to an activational effect of PRL, while paternal aggression, does not rely on PRL for its activation or organization.

DEDICATION

Oh! When will this
long weary day have end
and lende me leave
to come unto my love.
- E. Spenser

To my loving husband David. Even though we've been apart this year, this would not have been possible without your help and encouragement. Thank you. To the future!

ACKNOWLEDGEMENTS

I am grateful for the guidance of Dr. Matt, and the other members of my committee, Dr. Alcock and Dr. Kammer. In addition, I would like to thank the members of the lab: Les Castro, Greg Crever and Veronica Luna for their assistance. Finally, a thank you to Larry Neinbauer for all his insight and assistance with the animals.

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INTRODUCTION

This set of experiments explored the physiological regulation of male parental care in the Siberian dwarf hamster, Phodopus sungorus. Male Siberian dwarf hamsters nest with their young and are aggressive towards male intruders. From this, is seems paternal care is composed of two primary components: 1) behavior directed towards the pups, including nest building, cuddling, and grooming and 2) defensive behaviors directed towards intruders, including chasing, attacking, and fighting. As components of paternal care, it might be expected that these behaviors result from a common mechanism. Increased aggression in parental males, however, could be a response to the presence of a defendable stimulus (the female and her pups). In the first study, I tested whether this parental aggression was a stimulus response or a result of a more enduring mechanism by determining if parental males would be aggressive towards an introduced intruder in both the presence and absence of their pups. When the response to intruders was assessed, parental males were consistently and significantly more aggressive than control males, regardless of the presence or absence of the family group. Thus, paternal aggression appears to result from an enduring, physiological mechanism. The question then arises as to what regulates this change in behavior, is it hormonally induced? Evidence from other studies indicates

that plasma levels of prolactin are elevated in males of this species during the time of their mate's pregnancy. In order to determine if these changes in prolactin are important in programming paternal aggression, I injected males with an inhibitor of prolactin throughout the time period that they were paired with females and then quantified nurturing and aggressive behavior in these males in the presence of their young. Results indicate that in parental males with suppressed prolactin, the frequency of nurturing behavior is decreased but the frequency of paternal aggressive behavior remains elevated. Together, these studies strongly suggest that the two components of paternal behavior are regulated by two separate mechanisms. Component 1, or behavior towards the pups appears to be the result of an activational effect of prolactin. However, component 2, paternal aggression is a "hard-wired" behavior, that most probably consists of a neural program, which does not depend on plasma levels of prolactin for its activation.

CHAPTER 1

REGULATION OF PATERNAL AGGRESSION IN THE SIBERIAN DWARF HAMSTER, PHODOPUS SUNGORUS

ABSTRACT

In the Siberian dwarf hamster, parental males nesting with young are known to be more aggressive than unmated males. As a component of paternal care, defensive aggression may share a common regulating mechanism with the other behaviors that are associated with the parental state, such as nesting and cuddling. An additional explanation is that paternal aggression is a stimulus response to the presence of the female and pups. When the response to an intruder was assessed, parental males were consistently and significantly more aggressive than control males, regardless of the presence or absence of the family group. Thus, this behavior is not dependent on this social stimulus, but rather, appears to result from an enduring, physiological mechanism. The question then arises as to what regulates this behavioral change. Is it hormonally induced? Evidence from other studies suggests that paternal behavior is mediated by the hormone prolactin (PRL). Paternal aggression, however, probably does not result from an activational effect of PRL. Parents removed from the family group remained aggressive despite a significant PRL decrease. Such data demonstrate that this

aggressive or defensive component of paternal care may be regulated by a neural programming mechanism.

INTRODUCTION

Paternal care in rodents is often considered to be male participation in nurturing behaviors such as nesting, nest building and pup retrieval. The male parent may also serve other important functions, such as thermoregulation and defense from predators and conspecifics (see Elwood, 1983 and Dewsbury, 1985 for reviews). Thus, paternal care may actually consist of several behavioral components.

Defense from conspecifics may be an extremely important function of the parental male rodent because most unmated males are infanticidal and often succeed at killing pups despite the mother's defense (vom Saal, 1984). Defending the young against such rivals might require the parental male to be even more aggressive than his unmated counterpart.

Observations in our own laboratory have indicated that male Siberian dwarf hamsters (Phodopus sungorus) with young are significantly more aggressive toward a male intruder than are unmated males (Foster, Crever and Matt, 1990). Males of this species also nest with their young and perform care-giving behaviors (Gibber, Piontkewitz and Terkel, 1984; Sawrey, Baumgardner, Campa, Ferguson, Hodges and Dewsbury, 1984). Since the expression of increased aggression in Phodopus sungorus coincides with the

expression of care- giving behaviors, it may also be a parental behavior. Paternal care in this species may consist of at least two roles; one is nesting with and nurturing pups, and the other is defending pups from conspecifics.

If intermale aggression is a specific paternal behavior, it probably shares a common physiological mechanism with the care giving behaviors. Prolactin (PRL) is important in mediating the onset of paternal care in other vertebrates, particularly in fish and birds (see Brown, 1985 for review). Its function in male rodents, however, has been explored only recently. PRL has been positively correlated with male parental behavior in the California mouse (Peromyscus californicus), a species considered to be bi-parental (Gubernick and Nelson, 1989). In fact, both PRL levels and behavioral frequencies observed in males of this species are similar to those observed in females of this same species.

Observations in our own laboratory have also suggested that PRL is important in paternal care. The male Siberian hamster undergoes a gradual increase in plasma PRL during his mate's pregnancy, and PRL remains elevated throughout the care of young. Furthermore, the presence of young significantly delays the photoperiod induced decrease in PRL in these animals (Matt, Schoech and Morgan, 1990). In addition, when male hamsters are injected with a PRL

blocking agent, bromocriptine, the expression of the pup-directed behaviors is significantly reduced. The frequencies of aggressive behaviors, however, are not decreased by these injections, indicating that the physiological mechanism of paternal aggression may be independent of plasma PRL (Foster, Crever and Matt, 1990).

In male mice, aggression was nonspecifically stimulated by the presence of a "defendable resource," such as a shelter or an additional food source (McGregor, 1990), and the presence of young has also been shown to stimulate intermale aggression in a similar manner (Lore and Takahashi, 1984). Paternal aggression, therefore, may be a response to an immediate stimulus, rather than the product of a more enduring, physiological mechanism.

The first aim of the present study was to determine if the increase in intermale aggression that is associated with the paternal state is due the presence of a defendable stimulus (the female and pups). This was achieved by testing a parental male's response toward an intruder 24 hours after isolation from the family group. The second aim of the study was to determine if possible alterations in aggressive behavior could be due to changes in plasma levels of PRL.

METHODS

Experimental Design

Twenty-four virgin male Siberian dwarf hamsters (8 to 11 weeks of age) were paired with virgin females (7 to weeks 8 of age); eleven unpaired males (7 weeks of age) served as controls. All 35 males were ear-tagged for easy recognition during behavior testing. In addition, seven males, age-matched to the controls, were left untagged and used as intruders in behavior testing. All animals were born in our breeding colony at Arizona State University.

The breeding pairs were housed in small polycarbonate cages (28 x 17.5 x 13 cm); controls and intruders were housed as groups in large polycarbonate cages (48 x 26.7 x 12.7 cm). All cages were contained in rodent racks and provided with loose wood shavings for bedding. The animal facilities were maintained at a temperature of 23oC, relative humidity ranging between 20-60%, and a long day photoperiod (16 hours of light: 8 hours of darkness), with lights on at 0400 hours. Food (Purina Laboratory Chow 5001) and water were available ad libitum.

Twenty four hours prior to behavior testing, experimental pairs were matched for age of litter and divided into 2 groups: 1) parental males to be removed from the family group and transferred alone to large cages, n=12, 2) parental males to be transferred with the family group to the large cages, n=12. A third group, consisted of

unmated control males that were established individually in fresh, large cages, n=9. Experimental males were first bled, then transferred and left undisturbed for a 24 hour period.

Before behavior observations, experimental males were bled again. At the beginning of the observation period, a male intruder was introduced into the cage. Focal observations were done on the resident parental male or the resident control male for a ten minute period. The frequencies of ten behaviors were recorded for each minute of the test.

Pups were weaned at 21-23 days of age. Males rearing second litters were re-tested in the conditions that they did not experience in the first study (n=11). Blood samples were omitted in these second litter trials. Following these observations, animals were again restored to original cages.

When the males reached 124-141 days of age, the experiment was terminated. Males were killed by decapitation, and trunk blood was collected.

Blood Sampling

Throughout the experiment, all blood was collected by orbital sinus bleeds, using an unheparinized Pasteur pipette, without anaesthesia. Total handling time was less than one minute per animal. The collected blood was centrifuged at 3000 rpm at 4oC for 30 minutes, and plasma

frozen at -30oC until assayed for PRL.

Behavior Observations

Behaviors were recorded as they occurred, during each minute of the ten-minute test, and attack latency was recorded as the minute interval during which the initial attack occurred. Tests were terminated early when injury occurred to an animal. When an animal failed to attack, he was assigned a latency of 10. The observed behaviors are listed and described in Table 1.

Radioimmunoassay

Plasma PRL was measured in duplicates of 25 and 75ul using a homologous radioimmunoassay (Soares, Colosi and Talamantes, 1983) with iodinated hamster PRL and a specific antiserum against hamster PRL. Second antibody was a solid phase goat anti-rabbit gamma-globulin (Cal Biochem). All samples from this study were measured in one assay. The intra-assay coefficient of variation was 4.12%.

Statistics

Total aggressive scores for each animal were calculated post hoc on the basis of recorded behaviors using an unrotated Principal Components analysis. Components explaining greater than 10% of the variance were retained.

Intergroup comparisons of behavioral and hormonal variation were done using the Kruskall Wallis test. Specific intergroup comparisons of differences were then

analyzed using the Mann-Whitney U Test.

Effects of treatment were analyzed by comparing first and second litter data with the Paired Differences T test.

TABLE 1
Behaviors observed during the 10 minute test with an intruder male

1)	Attack	-	lunges, bites and fight initiations performed by the resident male.
2)	Fight	-	both males are active participants in an encounter involving lunges and bites.
3)	Chase	-	resident male actively pursues the intruder.
4)	Box	-	resident male alert on hind legs, thrusts with forelimbs.
5)	Wash	-	stroking fur with forelimbs, used in spreading scent from the ventral gland.
6)	Scent Marking	-	depressing ventral gland onto a surface.
7)	Vocalize	-	squealing or chirping noises.
8)	Scratch	-	stroking fur with hind paws.
9)	Dig	-	pawing or burrowing the bedding material.
10)	Sniff	-	male's nose contacts the intruder male.

RESULTS

Parental males with families present were more aggressive than unmated control males (see Figure 1). Attack latency was lower in parental males with families as compared to unmated control males ($P < .0012$), and parental males with their families present also had significantly greater frequencies of attacks ($P < .0067$), chases ($P < .023$), and fights ($P < .004$) when compared to unmated controls. In contrast, males with families boxed less frequently than unmated controls ($P < .027$).

Similarly, parental males without their families present also performed aggressive behaviors more frequently than unmated control males. Isolated parental males chased the intruder more frequently than did unmated control males ($P < .027$), and also showed reduced attack latencies ($P < .05$). Males isolated from their families showed elevated attack frequencies, but this difference was not significant ($P < .085$).

In contrast, parental males with families performed nonaggressive behaviors less frequently than unmated controls (Figure 2). Male parents with the family present did not sniff the intruder, or dig as frequently as unmated, control males ($P < .0074$ and $P < .0037$, respectively). There were no other significant differences between parent males with families and unmated control males in the other nondirected behaviors, including

washing, vocalizing, scratching and scent marking. Parent males isolated from their families were not significantly different from unmated control males in any of these nonaggressive behaviors.

There was great variability in the frequencies of each of the aggressive behaviors. In an attempt to quantify "aggressiveness," all behaviors measured in this study were subjected to a Principal Components Analysis. From this analysis we determined that total aggressiveness was best scored using attack, chase, fight, and wash frequencies; as well as inverse attack latency and inverse dig frequency. As illustrated in Figure 3, total scores generated from this analysis were significantly different among the three groups (Kruskall Wallis $P < .0018$). Like the scores for individual behaviors, the total aggressive scores of parental males with the family group present were greater than the scores of single, unmated control males ($P < .0004$). Isolated parental males also had greater aggressive scores than did single, unmated males ($P < .034$), but these were less than those of parent males with their families. Total aggressive score was not correlated with either plasma PRL ($P < .5$) or the age of the young ($P < .3$).

The PRL response to cage transfer differed among groups (see Figure 4). Following transfer to a new cage, male parents that had been transferred with their families,

as well as unmated control males, showed no change in plasma PRL. In contrast, parental males removed from their family for 24 hours showed a significant decrease in plasma levels of PRL (Paired T test $P < .018$).

There was a great deal of variability among individuals in the expression of parental aggressive behavior. In order to better estimate the effect of the testing paradigm, parental males that reared a second litter were retested in the regime that they did not experience during the first litter behavior test. There were no significant effects of the testing paradigm on total aggressive score (paired T test $P < .077$). Results suggest, however, that aggressive scores decrease in the absence of the family group (Table 2). Two animals did not respond to the intruder in either situation, and were excluded from all data analysis.

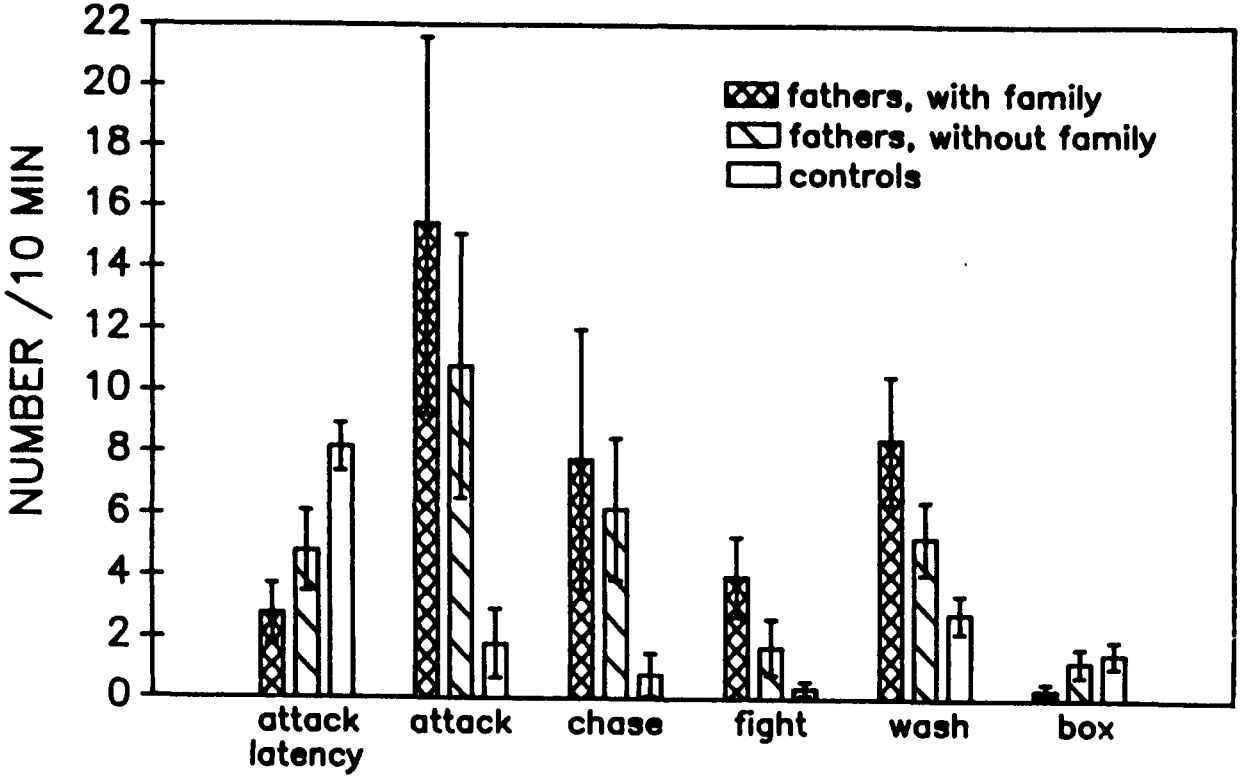

Fig 1. Number of times aggressive behaviors were performed by 1) parental male Siberian hamsters with the family group present, n=11 2) parental male Siberian hamsters without the family group present, n=11 , and 3) unmated, male Siberian hamsters, n=9, during a 10 minute trial with an intruder male.

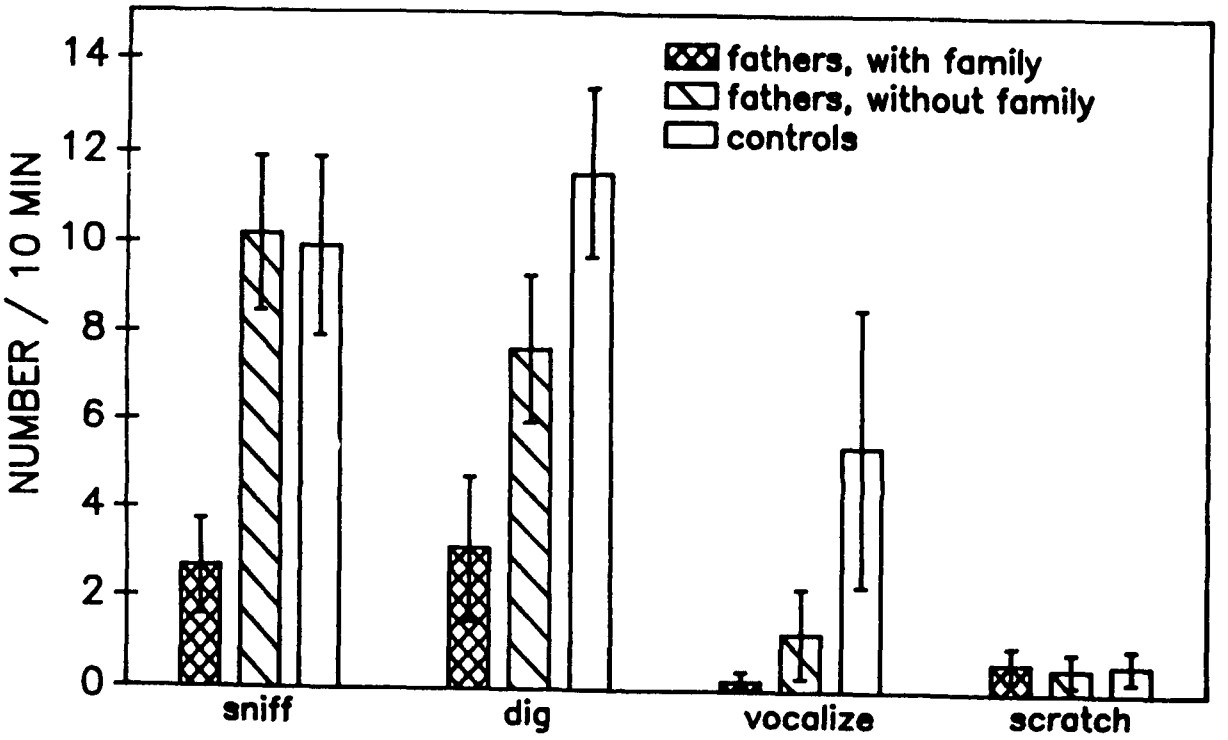

Fig.2. Number of times nonaggressive behaviors were performed by 1) parental male Siberian hamsters with the family group present, n=11, 2) parental male Siberian hamsters without the family present, n=11, and 3) unmated, male Siberian hamsters, n=9, during a 10 minute trial with an intruder male.

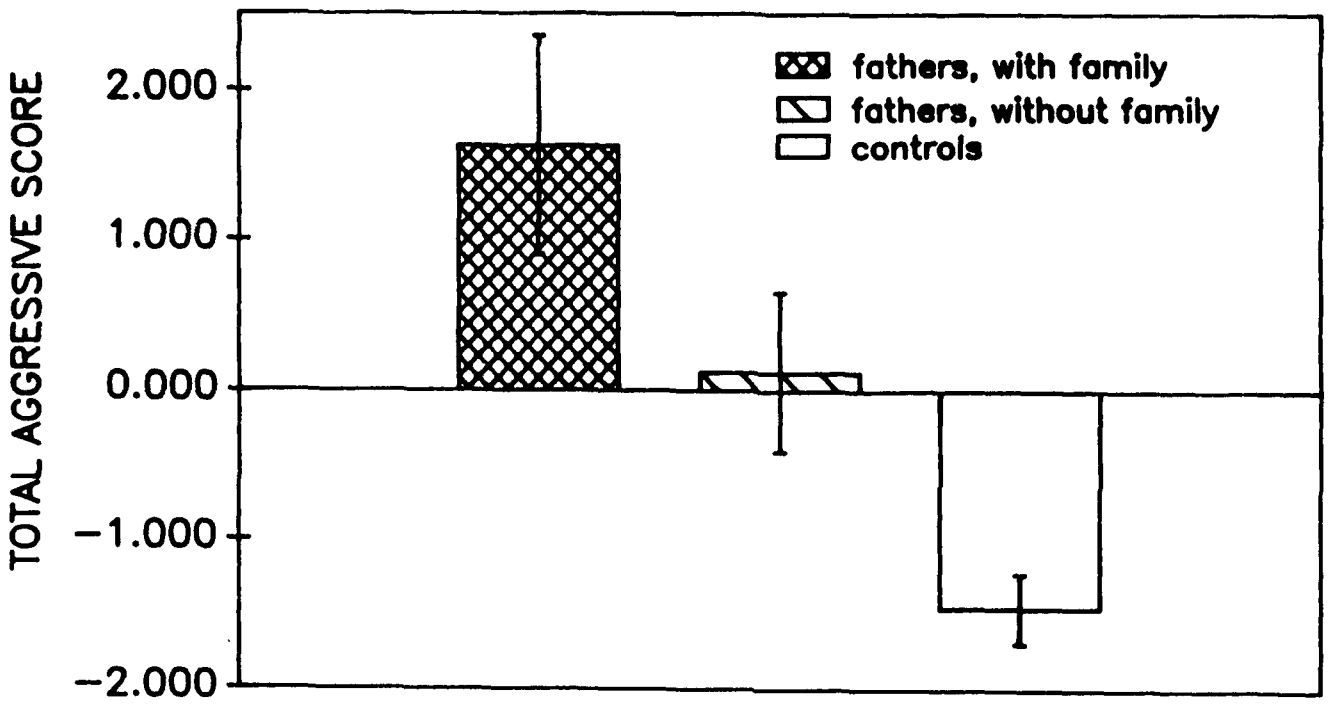

Fig. 3. Total aggressive scores for 1) parental male Siberian hamsters with the family present, n=11, 2) parental male Siberian hamsters without the family present n=11 , and 3) unmated, male Siberian hamsters n=9. Total scores were generated using a Principal Components Analysis.

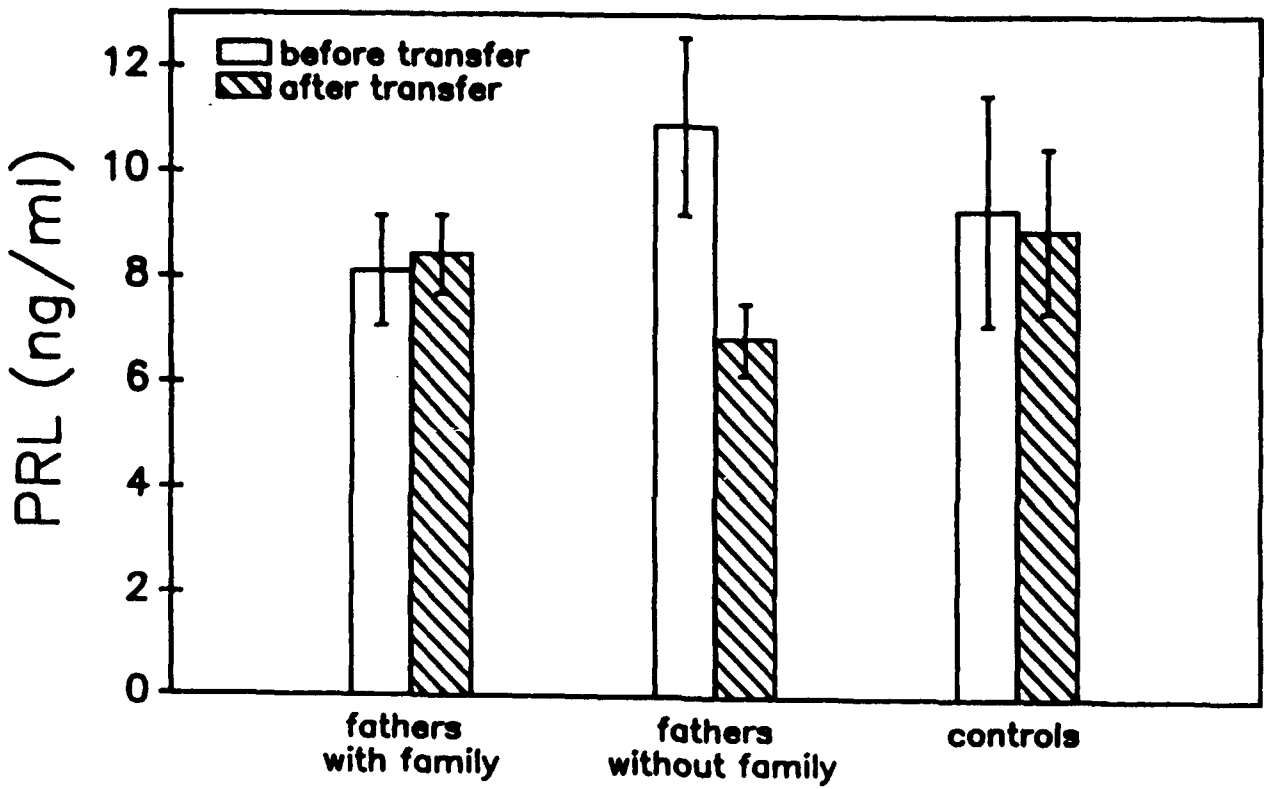

Fig. 4. Plasma PRL values before and after the 24 hour cage transfer in 1) parental male Siberian hamsters with the family present, n=11, 2) parental male Siberian hamsters without the family present, n=11, and 3)unmated, male Siberian hamsters n=9.

TABLE 2
A comparison of aggressive scores observed in opposing transfer conditions.

Males tested with the family present on the first litter trial were tested without the family group on the second litter. Males tested without families on the first litter trial were tested with them present on the second litter trial.

Animal No.	With Family Aggressive Score	Without Family Aggressive Score
2	0.8116	0.5853
8	3.3330	2.3254
12	-1.1243	-1.5340
13	1.6542	-1.8681
18	2.8938	-2.1380
19	1.0668	2.4646
20	3.2257	-0.1412
23	2.2541	1.9320
24	0.836	0.7031

DISCUSSION

The results of this study indicate that the intensified aggression exhibited by parental males is not dependent on the immediate presence of the family. In fact, the expression of paternal aggression in this study was relatively independent of both a defendable stimulus and plasma levels of PRL; it persisted despite changes in both of these parameters. This suggests that the basis of paternal aggression is a more durable physiological "program."

Several observations from this study suggest that the aggressive behavior of parental males is rooted in their physiology. First, parental males continued to show elevated aggression even when the female and pups had been removed for a 24 hour period. The aggressive scores of parental males were significantly greater than those of unmated, control males, regardless of the presence or absence of the female and pups (Fig. 1 and Fig. 3).

While the expression of paternal aggression does not depend on the immediate presence of the family, contact with the family does appear necessary to maintain paternal aggressiveness. The aggressive scores of males removed from their families were not as great as those of males with their families. So, although the behavioral "program" appears to exist autonomously, it may be further stimulated by cues from the young.

Some "fading" of paternal aggression is expected, particularly after long periods of time. Conspecific aggression is costly. If the female and / or pups were to meet with some disaster in the field, it would not be adaptive for a male to continue to remain intensely aggressive.

The distinctive and durable nature of paternal aggression is further evidenced by the differences between parental and unmated males in the latency to attack. Even in the absence of the family group, parental males required a much shorter period of time to approach and confront an intruder than unmated males. This was also reflected by the amount of time the males of each group participated in sniffing (Fig. 2). Unmated control males normally began encounters with a sniffing investigation of the intruder. Then, the resident control male usually either broke off the encounter or escalated to a biting attack. In contrast, parental males with their families present either did not investigate the intruder for as long, or attacked immediately, resulting in lower sniff frequencies. Even though parental males without their families present sniffed the intruder with a mean frequency similar to that of control males, they still exhibited a decreased attack latency. Parental males without their families approached the intruder more quickly to perform the sniffing investigation than did the unmated control males.

Paternal aggressive behavior was also distinguishable from the aggressive behavior of unmated males by the intensity and duration of encounters. For example, parental males continued pursuing and attacking, even upon receiving a passive signal from the intruder. Following an encounter with the resident male, the intruders in this study would often either lie on their backs, expose their ventral surfaces and vocalize or attempt to escape from the cage. After receiving one of these signals, control males ceased aggression more often than parental males in either group. Parental males often continued to attack the intruder while he was on his back, and also continued to pursue and attack the intruder throughout the duration of the test (personal observations).

Males that were with the female and pups spent significantly less time digging than unmated, control males (Fig. 2). This result arises because the males with families present were occupied in the specific act of defensive aggression throughout the testing period. Males engaged in defense performed these behaviors exclusively. In contrast, male parents absent from their families tended to dig with a frequency similar to that of control males. Again, paternal aggression may be augmented and maintained by contact with the young.

When parental males were tested in both the "with family" and "without family" paradigms, the aggressive

scores of males in the "without family" situation tended to be lower than those occurring in the "with family" situation (Table 2), although these were not significantly different.

The results presented in this study indicate that paternal aggression is probably not due to an activational effect of PRL. Parental males removed from their families underwent a significant decrease in plasma levels of PRL yet maintained high aggressive scores when compared to unmated males. Males that underwent the PRL decrease also retained greater frequencies of chasing and shorter attack latencies, when compared to unmated, control males. Likewise, Foster, Crever and Matt (1990) males that had been administered a PRL blocking agent significantly decreased the pup nurturing behaviors, but did not decrease aggressive behaviors toward an intruder.

It is likely that activation of aggressive behavior occurs prior to or coincides with the birth of the pups. Our results indicated that there was no correlation between the age of the young and the amount of aggression that was displayed by a parental male. The fathers of pups 1 day old were as aggressive as the fathers of pups 18 days old. Thus, paternal aggressive behavior is not a learned behavior.

If the function of the male is to defend his young from conspecifics, it would be most critical that this

behavior is present during the first few days of the pups' lives. During this time they are immobile and most vulnerable. Therefore, parental males must undergo some transformation from the unmated, less aggressive state to the parental, aggressive state, during gestation or partuition.

This transition "problem" is very similar to that associated with the onset of the other pup nurturing behaviors in male rodents. As mentioned previously, unmated males are normally infanticidal, and must reverse from pup- killing to pup-nurturing behavior. Like the intermale aggression of Phodopus sungorus, the expression of these behaviors has been observed to be independent of the amount of exposure the male has had to his young (vom Saal 1984). Transition from infanticidal to care giving behavior has been determined to take place during the pregnancy of the female by several observers. The trigger of this transition, however, is disputed and may be species- specific. vom Saal (1984) determined that copulation was sufficient to suppress infanticide in CS1 mice. Contradictory results, however, have been reported in the same species (Elwood and Ostermeyer 1984; Elwood, 1986). Soroker and Terkel (1988) reported that both copulation and cohabitation were necessary for the onset of parental behavior in wild mice. Elwood and Ostermeyer (1984) showed that cohabitation with the pregnant female

was the most important event associated with infanticide suppression in the Mongolian gerbil.

Recent findings indicate that these external triggers initiate physiological alterations which accomplish the transition from the unmated, infanticidal state to the parental care-giving state in male mice. The urines of infanticidal and parental male mice can be distinguished by mouse pups, suggesting that biochemical alterations are occurring in these male during the female's gestation period (Elwood, Kennedy, and Blakely, 1990).

The male Siberian hamster does undergo a notable physiological event during his mate's pregnancy. Throughout the gestation period, plasma PRL levels in males gradually increase, and then remain elevated throughout the care of young (Foster, Crever and Matt, 1990; Matt, Schoech and Morgan, 1990). PRL distinctions between parental males and unmated males were not observed in this study. However, blood sampling in this study was performed during the day, while samples in previous studies were taken during the dark cycle. In addition, PRL is released in a pulsatile manner and our samples may reflect trough as well as mean or peak values, masking the differences between groups.

PRL has been demonstrated to facilitate paternal care in several other species, particularly fish and birds

(Brown, 1985). This relationship has also been demonstrated in the California mouse, Peromyscus californicus (Gubernick and Nelson, 1989). Results in our own laboratory have shown that the presence of young can significantly delay the photoperiod induced decrease in plasma PRL, illustrating that in Phodopus sungorus the hormone may be important in the care of young (Matt, Schoech and Morgan, 1990). Foster, Crever and Matt (1990) demonstrated that blocking PRL significantly reduced the performance of parental care by male Siberian hamsters. Blocking plasma PRL did not, however, reduce the performance of aggressive behaviors directed toward an intruder. PRL in this study, however, was blocked just prior to testing, and not during the period of gestation in which this behavior appears to be activated.

Increased aggression appears to be a paternal function and its onset appears to coincide with an increase in plasma PRL in male Siberian hamsters during the mate's pregnancy. This suggests that this hormone is a likely candidate for the "internal trigger" of paternal aggression. It is probable that PRL acts organizationally during this time to facilitate a neural "program" for paternal aggression.

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CHAPTER 2

THE NEUROENDOCRINE CONTROL OF PATERNAL CARE IN THE SIBERIAN DWARF HAMSTER, PHODOPUS SUNGORUS

ABSTRACT

In the Siberian dwarf hamster, (Phodopus sungorus) paternal care consists of two components which are regulated by separate neuroendocrine mechanisms. Evidence suggests that the first component, which consists of pup-directed behaviors, results from an activational effect of prolactin. The second component, intruder-directed aggression, appears to be independent of plasma PRL, and may result instead from an organizational effect of the hormone achieved by an increase in plasma levels during the mate's pregnancy. To test these hypotheses, plasma PRL was suppressed in males throughout the time they were paired with a female, using bromocriptine (CB-154), a dopamine agonist. Suppression of plasma PRL in the male was effective in suppressing the pup- directed behaviors, but was not effective in suppressing the expression of aggression toward an intruder. These results confirm that the components of paternal care are regulated by separate mechanisms. PRL appears to definitely play an activational role in the expression of the pup-directed behaviors. The expression of intruder-directed aggression, however, does not appear to be either directly activated or organized by PRL.

INTRODUCTION

Male rodents may contribute to the survival of their offspring in several ways. The paternal actions observed most often, are the pup-caring behaviors such as nesting, cuddling, grooming and pup retrieval. Less-obvious paternal functions include thermoregulation and pup-defense (see Dewsbury, 1985, and Elwood, 1983 for reviews).

Male Siberian hamsters, Phodopus sungorus, nest with young, and are also significantly more aggressive toward male intruders than are unmated males (Foster, Crever and Matt, 1990). This suggests that paternal care in this species consists of at least two components. The first component consists of the pup-directed, nurturing behaviors previously described, such as nesting and cuddling. The second consists of aggressive behaviors directed against an intruder, and may serve the function of defense from conspecifics. Because both pup care and defense are components of paternal care, it might be expected that both share a common physiological mechanism. This, however, does not appear to be the case.

Paternal care is mediated by the hormone prolactin (PRL) in many vertebrates, particularly fish and birds (see Brown, 1985 for review). Recently, PRL has been correlated with paternal care in a rodent species, the California mouse, Peromyscus californicus (Gubernick and Nelson, 1989).

Observations in our lab also indicate that the pup-nurturing-behaviors are correlated with the hormone PRL. First, male Siberian hamsters with young have a significantly delayed decrease in PRL in response to a photoperiod change from long to short days (Matt, Schoech and Morgan, 1990). In addition, males whose PRL is suppressed while they are caring for young have significantly decreased frequencies of the pup-directed behaviors (Foster, Crever and Matt, 1990).

In contrast, increased aggression toward an intruder male remains unaltered despite acute decreases in plasma PRL. When fathers were removed from the stimulus of the female and pups for a period of twenty-four hours, aggressive behavior persisted despite a significant decrease in plasma levels of PRL (Murphy, 1991). Suppressing plasma PRL levels with a pharmacological agent also does not reduce the expression of aggressive behavior (Foster, Crever, and Matt, 1990).

Paternal aggression does not appear to be achieved through learning either. Aggressive scores of fathers were independent of the age of their pups, meaning that mediation of this behavior either occurs prior to or at the time of partuition (Murphy, 1991). In addition, paternal aggression is not the result of a stimulus response caused by the presence of the pups. Parental male Siberian hamsters were significantly more aggressive than unmated

males even when were removed from the presence of the family group for twenty-four hours (Murphy, 1991). The enduring nature of paternal aggressive behavior suggests that this component of paternal care is mediated through a neuroendocrine "program."

"When" and "how" this program originates remains to be determined. The onset of paternal care in rodents is characterized by suppression of infanticide and is closely associated with the gestation period of the female, although the exact "trigger" of this transition is disputed. Both copulation and cohabitation with a female have been implicated as events significant to the onset of paternal care. Of these, the latter seems to be the most important. For example, copulation significantly reduces the frequency of infanticide in male CS1 mice, but does not completely eliminate it. If the male remains with his mate, this effect is much more enduring. This illustrates that copulation is an instrumental experience in this transition, but is not solely responsible for it (Elwood, 1986). In fact, if virgin males are housed with a pregnant female, they also make the transition to a non-infanticidal state. In the Mongolian gerbil, cohabitation with the female is crucial to the inhibition of pup cannibalism and the onset of paternal behavior (Elwood and Ostermeyer, 1984). A programming mechanism for paternal aggression might also coincide with the pregnancy of the mate.

Throughout the period of their mate's pregnancy, Phodopus sungorus males undergo a gradual increase in plasma levels of PRL, which then remain elevated throughout the care of young (Matt, Schoech and Morgan, 1990). Although PRL does not act activationally to mediate paternal aggressive behaviors, this increase in PRL during the mate's pregnancy may still act organizationally to create a neurophysiological "program."

Therefore, I propose that the components of paternal care in the Siberian dwarf hamster are regulated by distinct, but intimately related mechanisms. Specifically, the pup-directed behaviors result from an activational effect of PRL, and the intruder-directed, aggressive behaviors result from a direct organizational effect of PRL. This organizational effect of PRL would be achieved through the increase in plasma PRL that occurs in the male throughout his mate's pregnancy.

METHODS

Experimental Design

In order to test the hypotheses that plasma PRL activates pup-directed behaviors and organizes intruder-directed behaviors, we designed the experiment with the purpose of suppressing plasma PRL in males throughout the time they were paired with a female. This was done in order to prevent the gradual increase in PRL, which is potentially organizational to paternal aggression. PRL was

suppressed using bromocriptine (CB-154), a dopamine agonist. As a control, additional groups of animals received injections of the sesame oil vehicle.

24 virgin male Siberian dwarf hamsters (8 to 12 weeks of age) were paired with 24 virgin females (6 to 8 weeks of age). These paired males, as well as 20 unpaired males (9 to 13 weeks of age) were divided among four treatment groups: 1) paired males receiving injections of CB-154 (n=12); 2) paired males receiving injections of the oil vehicle (n=12); 3) unmated males receiving injections of CB-154 (n=10); 4) unmated males receiving injections of the oil vehicle (n=10).

All animals were born in our breeding colony at Arizona State University. Breeding pairs were housed in small polycarbonate cages (28 x 17.5 x 13 cm). Unmated control males and intruders were housed as groups of 5 in large, polycarbonate cages (48 x 26.7 x 12.7 cm). All cages were contained in a rodent rack and provided with loose wood shavings for bedding. Food (Purina Laboratory Chow 5001) and water were available ad libitum. The animal facilities were maintained at a temperature of 23^{o}C, relative humidity between 20 and 60%, and a long day photoperiod (16 hours of light, and 8 hours of darkness) with lights on at 0400 hours.

Blood samples were taken from the paired males and unmated controls at baseline point, during the mate's

pregnancy, and 48 hours prior to behavior testing. Behavior observations were conducted when pups were 9-11 days of age. Males were killed and trunk blood was collected when the pups were weaned at 18-21 days of age.

Injections

Subcutaneous injections of bromocriptine (Sandoz Pharmaceuticals) were performed daily, between 0800 and 1030 throughout the entire experiment. Bromocriptine dose was
0.4mg CB-154/.04ml sesame oil/animal/day. Control animals were injected with this same volume of sesame oil (.04ml oil/animal/day).

Blood Sampling

To monitor PRL values throughout the course of the experiment, blood samples were collected from males at baseline, during the mate's pregnancy, when the pups reached 7-9 days of age (48 hours prior to behavior observations, and when the pups were weaned at 18-22 days of age. A matched number of unmated controls were bled each time samples were collected from parental males. Blood was collected by orbital sinus bleeds using an unheparinized Pasteur pipette, without anaesthesia. Total handling time was less that one minute per animal. Fathers were killed by decapitation when their pups reached 18-22 days of age. A matched number of unmated control males

were also killed at this time. Trunk blood, testes and seminal vesicles were collected. The collected blood was centrifuged at 3000 rpm at 4°C for 30 minutes, and the plasma was frozen at -30°C until assayed for PRL. Seminal vesicle and testes weights were recorded.

Behavior Observations

All experimental males were tagged in the right ear for easy recognition during behavior testing. Ten untagged males (13 to 15 weeks of age) were used as intruders in behavior experiments.

Behavior observations were performed between 0830 and 1400 hours when the pups were 9-12 days of age. 24 hours following the blood sample (collected at 7-9 days of age), all animals were transferred to experimental conditions. Pairs were transferred to large cages with their litters. Unmated males were established individually in fresh, large cages, and animals were left undisturbed for an additional 24 hours.

The following morning, paired males were first observed for a ten minute parental trial, during which the total time in the nest and the frequency of 12 behaviors were recorded for each minute interval (see Table 1).

All males were subjected to the ten minute intruder test. Behaviors were recorded as they occurred, during each minute of the ten-minute test (see Table 2). Attack latency was recorded as the minute interval during which

the initial attack occurred. When an animal failed to attack, he was assigned a latency of 10. Intruder tests were terminated early when injury occurred to an animal. All animals were returned to their home cages following behavior testing.

Radioimmunoassay

Plasma PRL was measured in duplicates of 25 and 75ul using a homologous radioimmunoassay with iodinated hamster PRL and a specific antiserum against hamster PRL (Soares, Colosi and Talamantes, 1983). Second antibody was solid phase goat anti-rabbit gamma immunoglobulin (heavy and light chain) (Cal Biochem # 170-5602).

Statistical Analysis

The frequency with which parental behaviors were observed in this study was relatively low. Therefore, parental behavior was scored in two separate forms in this study. First, the total number of pup contacts was determined by adding scores for nesting, cuddling, pup grooming, pup sniffing and huddling.

Aggressive behaviors observed in this study were subjected to an unrotated Principal Components Analysis. Components explaining greater than 10% of the variance were retained. A resulting component was used to generate total aggressive score. This was significantly weighted by attack, chasing, fighting and inverse attack latency.

Intergroup comparisons of behavior and hormone data were done using the Kruskall Wallis test. Specific comparisons were then analyzed with the Mann-Whitney U test. Organ weights were compared using the independent groups T test.

TABLE 3
Behaviors observed during the 10 minute parental trial.

1)	Nest building	- male carries nesting material in mouth and deposits on top of nest.
2)	Nest	- male enters nest where pups are located.
3)	Washing	- male stokes fur with hindlimbs.
4)	Groom	- male grooms self with mouth.
5)	Scratch	- male stokes fur with hindlimbs.
6)	Dig	- pawing or burrowing in bedding material.
7)	Sniff pup	- male's nose moves over pup(s).
8)	Groom pup	- male licks pup(s).
9)	Cuddle	- male curled around pups.
10)	Huddle	- male crouches over and covers pups.

TABLE 4
Behaviors observed during the 10 minute test with an intruder male.

1)	Attack	- lunges, bites and fight initiations performed by the resident male.
2)	Fight	- both males are active participants in an encounter involving lunges and bites.
3)	Chase	- male actively pursues the intruder.
4)	Box	- male alert on hind legs, thrusts with forelimbs.
5)	Wash	- stroking fur with forelimbs, used in spreading scent from the ventral gland.
6)	Scent Mark	- depressing ventral gland onto a surface.
7)	Vocalize	- squealing or chirping noises.
8)	Scratch	- stroking fur with hind limbs.
9)	Dig	- pawing or burrowing in bedding material.
10)	Sniff	- male's nose contacts the intruder male.

RESULTS

The bromocriptine treatment administered to males in this study was effective in suppressing plasma levels of PRL. Figure 1 illustrates that plasma PRL was significantly reduced at the blood sampling 48 hours prior to the time of behavior testing (pups 7-9 days of age); for comparison between CB-154 and oil treated parental males, $P < .005$, and for comparison between CB-154 and oil-treated unmated males, $P < .0009$. Plasma PRL was also suppressed in males during their mate's pregnancy (Fig. 2); for comparison between CB-154 and oil treated parental males, $P < .0167$, and for comparison between CB-154 and oil treated unmated males, $P < .0006$.

The parental males with suppressed PRL (CB-154 treated males) performed significantly fewer parental behaviors than did their oil-treated counterparts. As illustrated in Fig. 3, fathers treated with CB-154 performed significantly fewer pup contacts than did fathers treated with oil ($P < .023$).

Figure 4 indicates oil-treated fathers performed aggressive behaviors more frequently than did oil-treated unmated males. Fathers injected with oil had significantly reduced attack latencies when compared to unmated males injected with oil ($P < .05$). Fathers receiving oil also

scored significantly higher in attacks ($P < .033$), and chases ($P < .04$), than did unmated males receiving oil.

Bromocriptine treatment did not change the expression of aggressive behaviors in parental males. There were no significant differences between fathers injected with CB-154 and fathers injected with oil in attack latency, or scores of attacks and chases.

Additionally, There were no significant differences in attack latencies or scores of attacks and chases between CB-154-injected and oil-injected unpaired males.

A listing of other aggressive behavior scores is reported in Figure 5. Oil-injected fathers had significantly greater frequencies of fighting ($P < .021$) and washing ($P < .032$) than oil-injected, unmated males. Again there were no significant differences between oil-injected and CB-154-injected fathers, or between oil-injected and CB-154-injected unmated males.

Total aggressive scores were significantly different between oil-injected fathers and oil-injected controls ($P < .006$). However, there were no significant differences between fathers injected with CB-154 and fathers injected with oil. Likewise, there were no significant differences between the total aggressive scores of unmated males injected with CB-154 and unmated males (Fig. 6).

Finally, oil-injected fathers had significantly greater seminal vesicle weights than did unmated males

injected with oil ($P < .002$) Fig. 7). There was no significant difference between the seminal vesicle weights of fathers injected with CB-154 and fathers injected with oil. Similarly, oil- injected unmated males were not significantly different from CB-154-injected unmated males in seminal vesicle weights, however, there was a trend for CB-injected controls to have heavier seminal vesicles ($P < .074$).

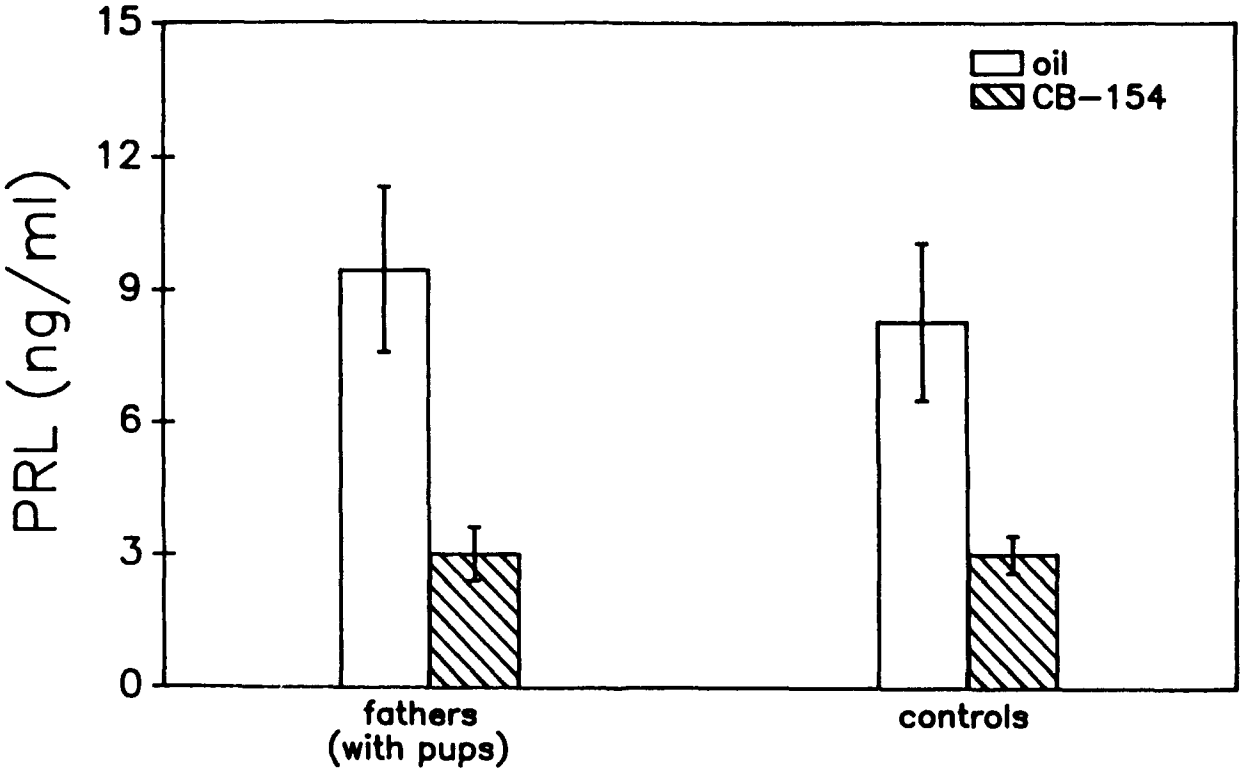

Fig. 5. Plasma PRL values of male Siberian hamsters 48 hours before behavior testing. Males had received one of 4 treatments 1) parental males, oil injections, n=11, 2) parental males, CB-154 injections, n=8, 3) unmated males, oil injections, n=10, 4) unmated males, CB-154 injections, n=10.

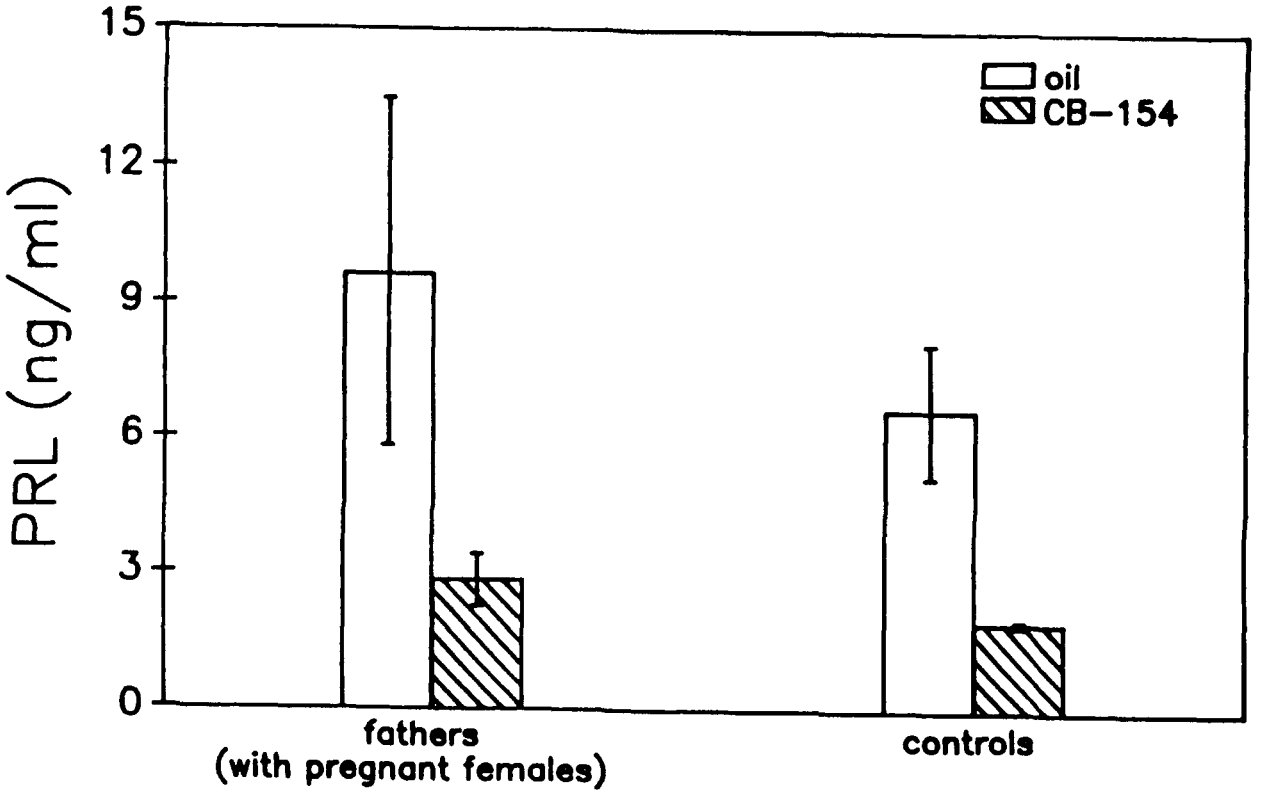

Fig. 6. Plasma PRL values of male Siberian hamsters during their mate's pregnancy. Males had received one of 4 treatments 1) parental males, oil injections, n=11, 2) parental males, CB-154 injections, n=8, 3) unmated males, oil injections, n=10, 4) unmated males, CB-154 injections, n=10.

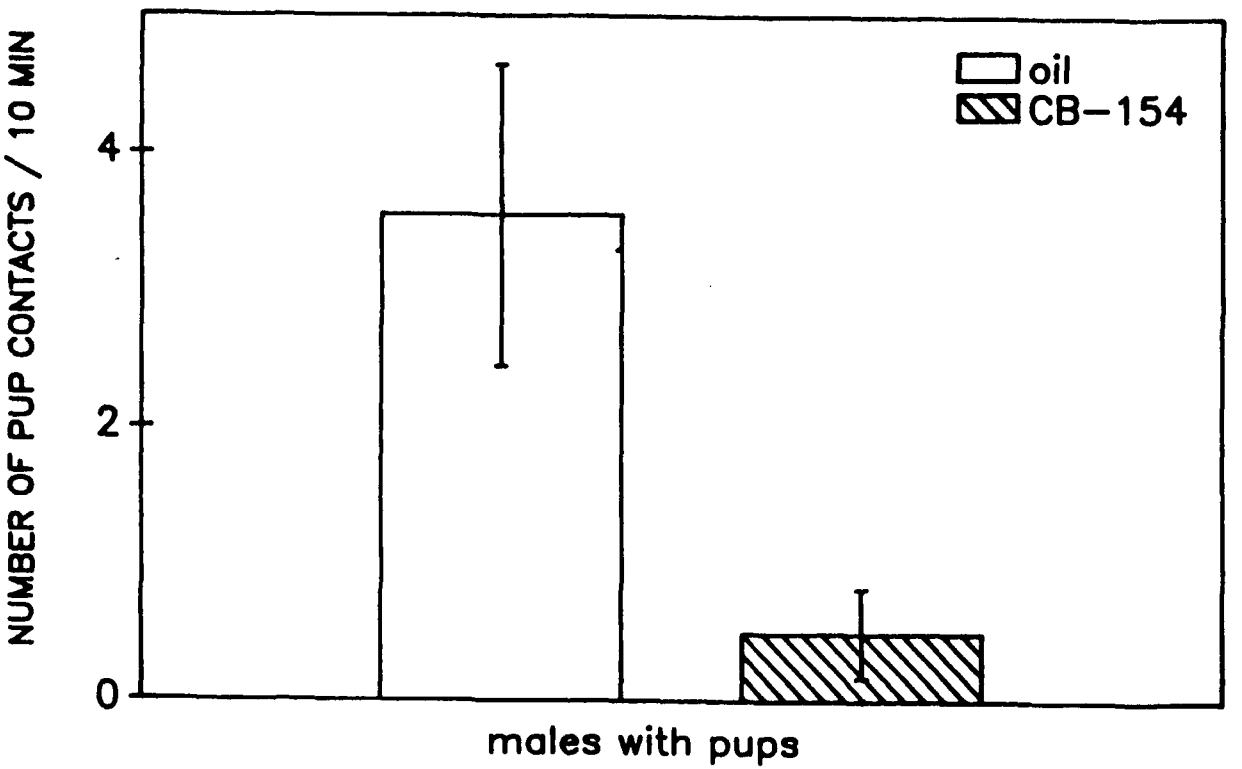

Fig. 7. Number of times oil-injected parental male Siberian hamsters, n=11, and CB-154-injected parental male Siberian hamsters, n=8, contacted their pups during the 10 minute parental test.

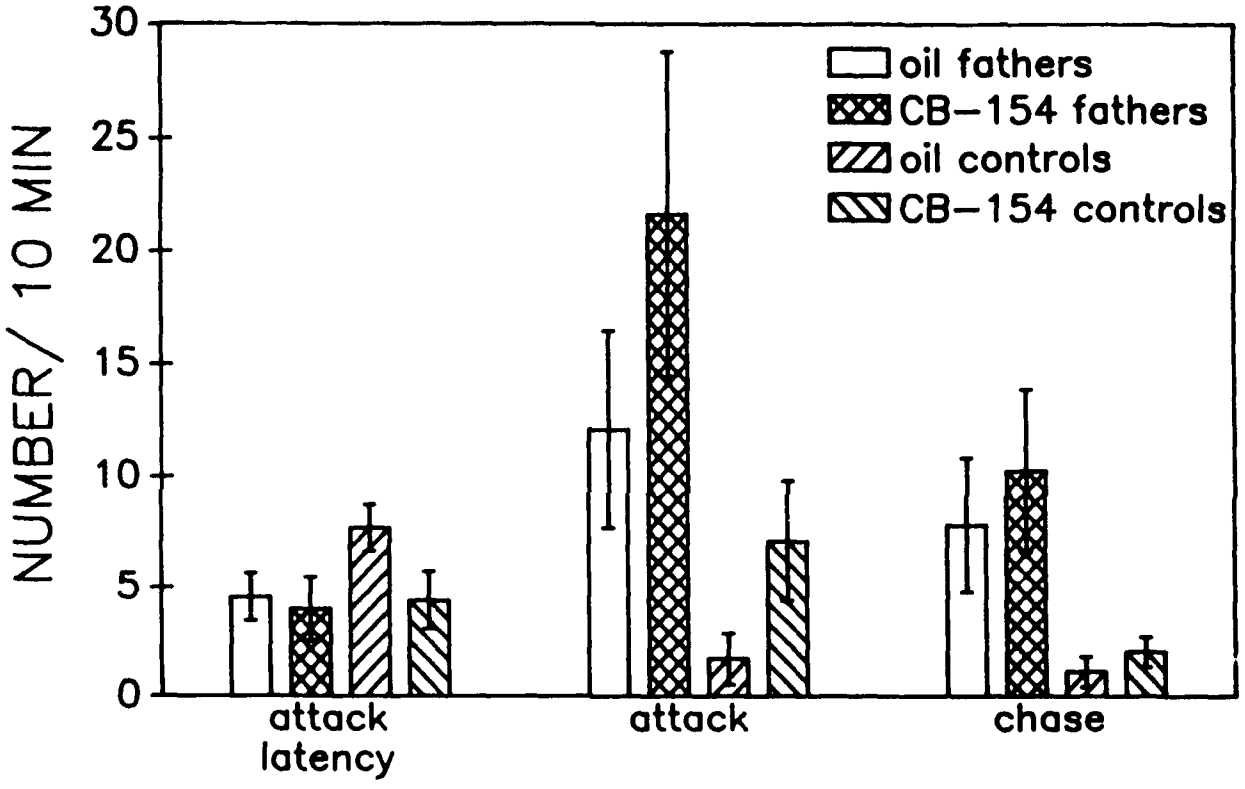

Fig. 8. Number of times aggressive behaviors were performed by male Siberian hamsters in a 10 minute trial with an intruder male. Males had received one of 4 treatments 1) parental males, oil injections, n=11, 2) parental males, CB-154 injections, n=8 3) unmated males, oil injections, n=10, 4) unmated males, CB-154 injections, n=10 .

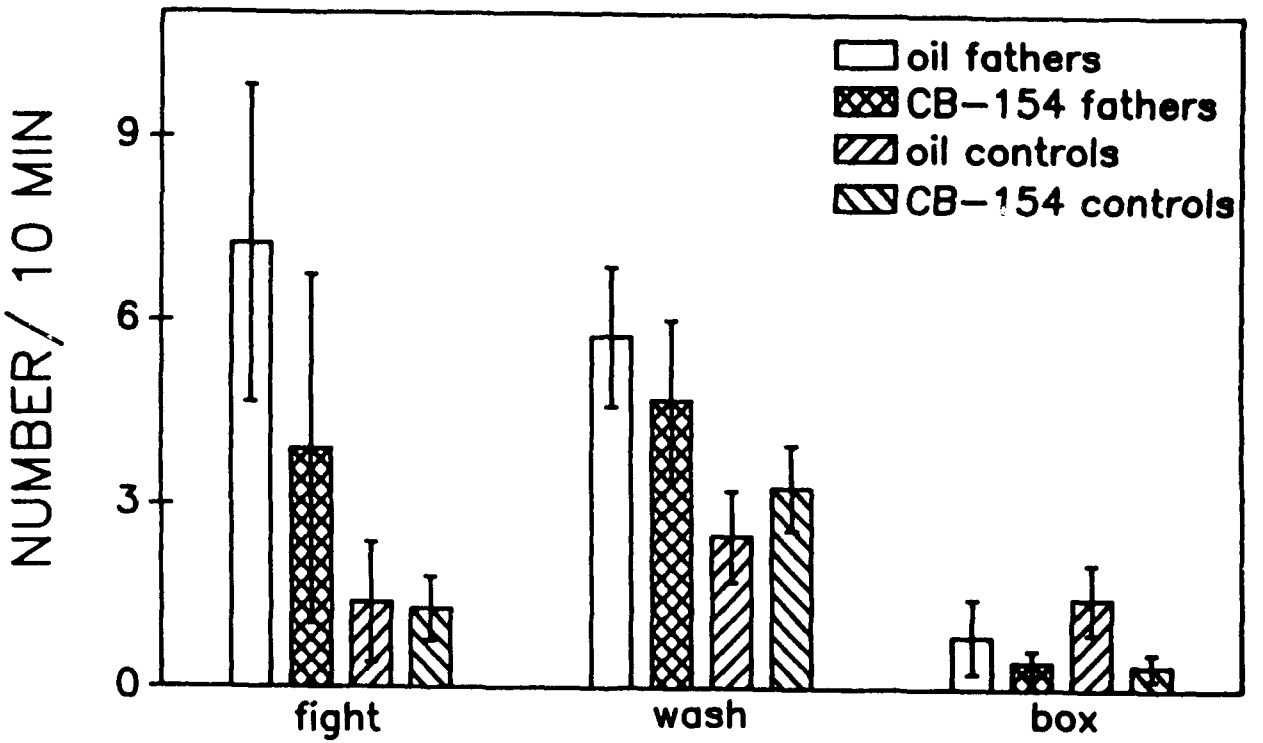

Fig. 9. Number of times aggressive behaviors were performed by male Siberian hamsters in a 10 minute trial with an intruder male. Males had received one of 4 treatments 1)parental males, oil injections, n=11, 2) parental males, CB-154 injections, n=8, 3) unmated males, oil injections, n=10, 4) unmated males, CB-154 injections, n=10.

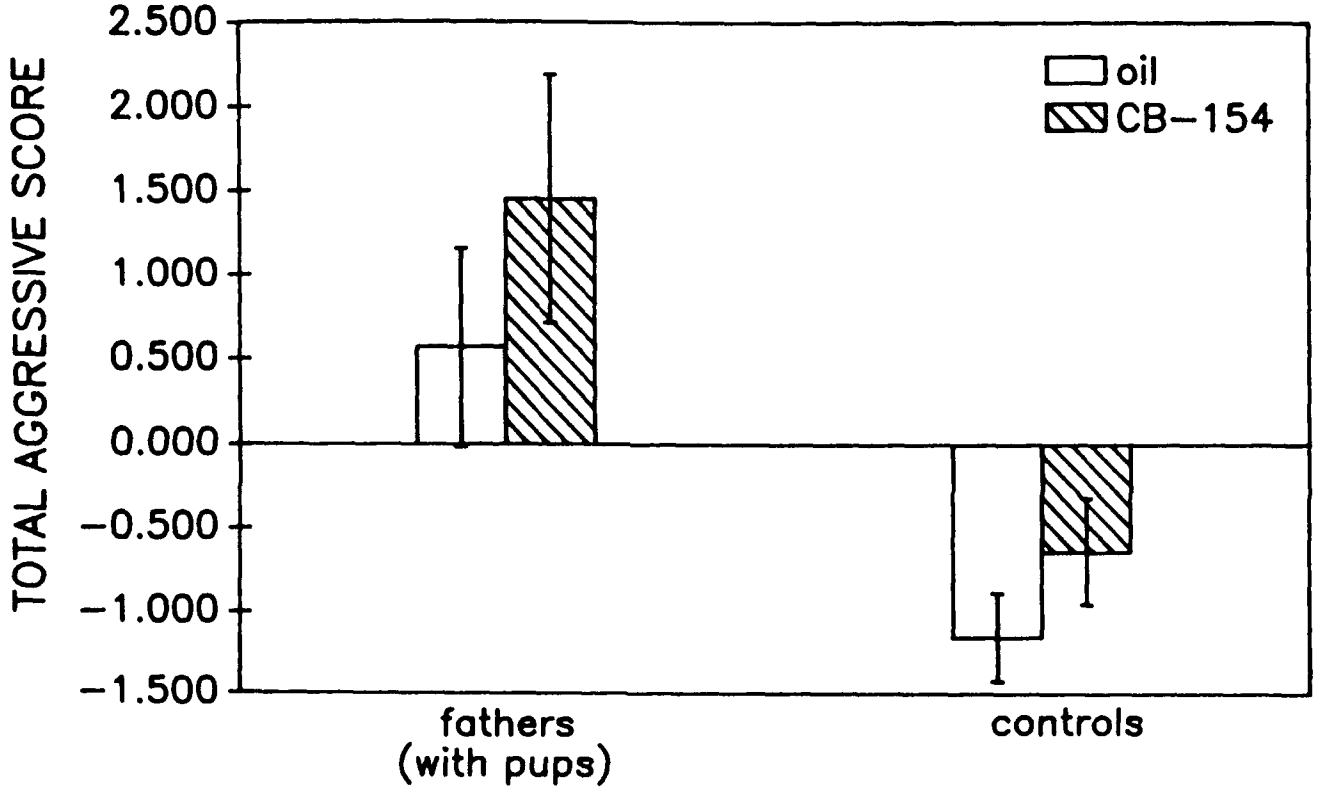

Fig. 10. The total aggressive scores of male Siberian hamsters. Males had received one of 4 treatments 1) parental males, oil injections, n=11, 2) parental males, CB-154 injections, n=8, 3) unmated males, oil injections, n=10, 4) unmated males, CB-154 injections, n=10. Total aggressive score was generated using a Principal Components Analysis.

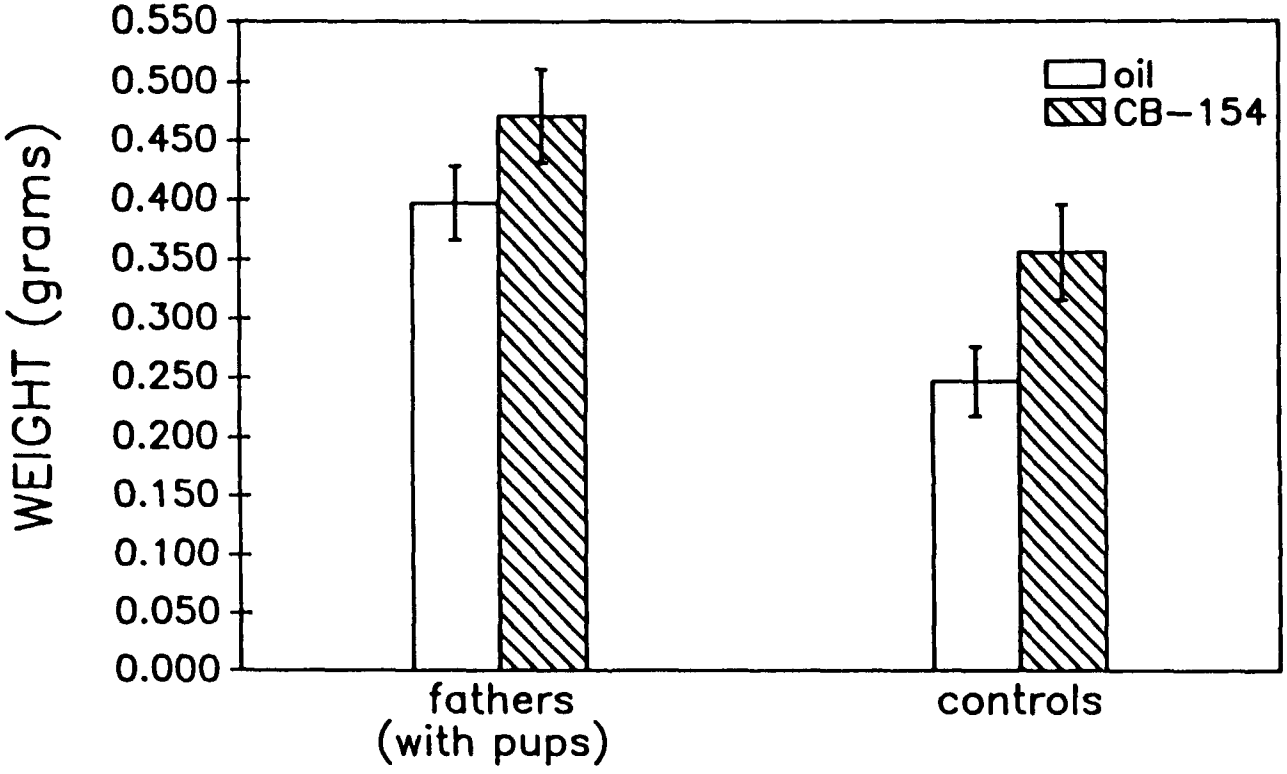

Fig. 11. Seminal vesicle weights of male Siberian hamsters. Males had recieved one of 4 treatments 1) parental males, oil injections, n=11, 2) parental males, CB-154 injections, n=8, 3) unmated males, oil injections, n=10, 4) unmated males, CB-154 injections, n=10.

DISCUSSION

The results of this study indicate that the two components of paternal care observed in the Siberian dwarf hamster are controlled by separate mechanisms. The first component, which consists of the pup-nurturing behaviors such as nesting and cuddling, is mediated by an activational effect of PRL. The second component, defensive aggression toward intruders, appears to be mediated by a neurophysiological "program" which does not rely on PRL for its activation or organization.

Observations from this study confirm that male care of young in the Siberian hamster is a PRL-dependent behavior. Parental males that received CB-154 injections had significantly reduced plasma levels of PRL. These animals made significantly fewer total contacts with their pups than did parental males that received injections of only sesame oil (Fig. 3). Total pup contact is a composite score which consists of sniffing the pups, grooming the pups, cuddling with the pups in the nest, and huddling over the pups. Thus, reduced pup contact score means that male parents with suppressed PRL performed significantly fewer care giving behaviors than did male parents with unmanipulated PRL.

Nesting was also suppressed in parental males injected with CB-154 as compared to oil-treated controls, although this difference was not significant. Nesting may be an

extremely important paternal behavior in P. sungorus, because the male's presence may be necessary as a thermoregulatory contribution. These animals inhabit the steppes of Siberia, where they experience mean low temperatures of -25°C. In an experiment where pairs of P. sungorus and pairs of the closely related species P. campbelli where subjected to temperatures of 4°C, the presence of both parents was sufficient to maintain pup survival in P. sungorus, but not in P. campbelli (Wynne-Edwards and Lisk, 1988).

The results of this study indicate that depressing PRL severely depresses care giving behavior. Perhaps this occurs because PRL is activational, rather than organizational to these behaviors since administering CB-154 only just prior to behavior testing is also sufficient to depress the expression of pup-directed behaviors (Foster, Crever and Matt, 1990). Thus, the initial increase of plasma PRL does not seem sufficient to mediate the nurturing behaviors, but instead, the immediate presence of plasma PRL is required for their expression.

In contrast, the immediate presence of plasma PRL is not required for the expression of the second component of paternal care, defensive aggression toward an intruder. Parental male hamsters are significantly more aggressive toward a male intruder than unmated males. This distinctive behavior persists even when plasma PRL is acutely

decreased. When male parents undergo a significant PRL decrease upon removal from their family, their aggression toward an intruder is not significantly altered (Murphy, 1991). When Foster, Crever and Matt (1990) achieved an acute decrease in plasma PRL using CB-154, the aggressive response to a male intruder was, again, not diminished.

Intruder-directed aggression is, however, intimately associated with the paternal state. This is illustrated by the fact that fathers are significantly more aggressive than unmated males (Foster, Crever and Matt, 1990; Murphy, 1991). Due to this, it would be expected that the mechanism responsible for the expression of paternal aggression was closely linked to that of the paternal pup-directed behaviors. These are, as mentioned previously, mediated by an activational effect of PRL.

The highest levels of PRL observed in parental males in this study was during their mate's pregnancy (Fig 2). Matt, Schoech and Morgan (1990) have reported this same pattern of plasma PRL levels. Perhaps the initial increase in plasma levels of PRL that the male undergoes during the female's pregnancy acts organizationally to "program" the expression of paternal aggression. A neural "program" organized by the increase in plasma levels of PRL the male during his mate's pregnancy would explain the persistence of aggression despite decreases in plasma PRL

levels and the removal of the family group stimulus (Murphy, 1991).

The results of this study, however, indicate that this increase in plasma levels of PRL in the male during his mate's pregnancy is not organizing the increase in intermale aggression exhibited by parental males.

PRL suppression during the female's pregnancy does not change the expression of any of the aggressive behaviors (Fig. 5 and Fig 6). Fathers whose PRL was suppressed throughout the time they were paired with a female, again actually appeared to score slightly higher than fathers whose PRL was not manipulated. The number of attacks and chases performed throughout the 10 minutes with an intruder male is greater in fathers injected with CB-154 than fathers injected with oil, although this difference is not significant.

In addition, the total aggressive scores of parental males whose plasma PRL was suppressed throughout the female's pregnancy (CB-154-injected fathers) were not lower than those of parental males with unaltered PRL (oil-injected fathers). In fact, the scores of fathers receiving CB-154 appear slightly higher than do those of fathers receiving only oil.

From these results I conclude that the increased aggression observed in parental males is not produced by either a direct activational or organization effect of PRL.

This, however, does not exclude the possibility that PRL may participate in organizing this behavior in an indirect manner. PRL secretion is regulated by the neurotransmitter dopamine (DA), which tonically inhibits its release. Thus, if one considers a regular, negative feedback loop, increases in plasma PRL would result in increased DA activity (Kordon et. al., 1985). In Phodopus sungorus, males paired with females and young have increased dopamine content in the medial basal hypothalamus (Matt, Schoech and Morgan, 1990). The increase of plasma PRL levels during pregnancy, therefore, may serve to initiate an increase in dopamine DA activity of the hypothalamus or other areas of the brain. Increased DA activity in the limbic system, in particular, may induce aggression (McKim, 1986).

If PRL does "program" paternal aggression through dopamine, the use of a dopamine agonist (CB-154) would have actually mimicked the action of PRL. That is, the agonist itself might produce the "program." This is further substantiated by the fact that animals that were administered bromocriptine in this study actually exhibited increased aggression, rather than decreased aggression. For instance, fathers receiving CB-154 have actually slightly greater attack and chase scores than fathers that were treated with only oil (Fig. 3). In addition, unmated males that received CB-154 injections also show slight elevations in attack and chase; these males also have

shorter latencies to attack than unmated males treated with oil. Thus, it appears that there may have been a drug effect on aggression during this study. The levels of aggression observed in unmated males that were administered CB-154, however, still do not approach those of parental males (Fig. 3 and Fig. 4).

The unique specific nature of paternal aggression suggests that PRL may interface with another hormone. The aggression displayed by parental male Siberian hamsters is unique because while these males are more aggressive toward a male conspecific, they do not commit infanticide, or attack the female. When PRL is suppressed in parental male Siberian hamsters, the expression of aggression becomes less specific, and parental males will also attack the female and pups (Foster, Crever and Matt, 1990). Normally, aggression in rats is correlated with the hormone testosterone (T) (Albert, Dyson, Walsh and Petrovic, 1988).

Studies of maternal behavior have shown that PRL mediation is not possible unless the female is first steroid- primed (Bridges, et. al., 1985; Kinsley and Bridges, 1988). A similar interaction may be responsible for paternal aggression which involves T and PRL. In this study, seminal vesicle weights, which are testosterone dependent, are significantly greater in parental males than in unmated males (Fig. 7). In males of P. sungorus paired with females, T rises significantly throughout the time the

male is with the female, peaks at proestrus and is significantly decreased at postpartum estrus (Schoech and Matt, 1990). This pattern of testosterone secretion may be the mechanism by which the male is steroid primed.

PRL may then act on this "template." In this study, the seminal vesicle weights of CB-154-injected males tended to be greater than those of oil-injected males, however, this difference was not significant. PRL has been demonstrated to be instrumental in decreasing T sensitivity of the hypothalamo-pituitary axis after photostimulation so that long day photoperiod levels of gonadotropins, and hence, the reproductive state may be maintained (Matt, et. al., 1984). PRL may also be instrumental in decreasing the parental male's sensitivity to testosterone and thus, aggressiveness toward his own young. So, although PRL alone does not appear to organize paternal aggressive behaviors, it may still interface with T to direct paternal aggression.

In summary, the two different components of paternal care that are observed in the male Siberian dwarf hamster appear to be regulated by distinctly separate mechanisms, rather than through two different actions of the hormone PRL. Pup-directed behaviors are dependent on an activational effect of plasma PRL. Intruder-directed aggressive behaviors appear to result from a neurophysiological program, which does not rely on PRL for

its activation or organization. PRL may, however, participate in a more complex interaction, with the neurotransmitter dopamine or the hormone testosterone to mediate this unique, specific, aggressive behavior.

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